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Morphogenesis During Asexual Reproduction in *Pygospio elegans* Claparede (Annelida, Polychaeta)

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Abstract. The spionid *Pygospio elegans* reproduces both asexually and sexually. Using scanning electron and bright field microscopy, we examined morphogenesis following asexual reproduction to determine how "lost" body regions were regenerated after a worm spontaneously divided. Asexual reproduction occurred through transverse fission and divided the parent worm into 2 to 6 fragments (architomy). All fragments retained their original anterior-posterior polarity. Regeneration in all fragments followed a specific series of events: wound healing (day 1); extension of the blastema to generate lost body regions—specifically, the head and thorax for posterior fragments and the tail and pygidium for anterior fragments (days 2–3); segmentation (days 3–6); and differentiation of segment- or region-specific structures (days 4–8). This pattern occurred regardless of where the original division took place. Subsequent growth occurred through addition of terminal setigers anterior to the pygidium followed by differentiation of tail setigers into abdominal setigers, leaving the tail region about 6 to 10 setigers in size. Division rates were compared in worms from three populations in Nova Scotia, Canada. Worms from two populations (Conrad's Beach, Starr's Point) divided more frequently (about 1.2 and 1.3 weeks between divisions, respectively) than worms from Bon Portage Island (3.5 weeks between divisions). Fragments containing the original head (original mouth intact, generally much larger fragment) had a higher survivorship than fragments containing the original tail.

Introduction

Asexual reproduction is the process of forming two or more offspring from one parent body without involving gametes, or cells with a meiotically reduced chromosome number (Balinsky, 1975; Solomon *et al.*, 1993). Although polychaetes reproduce sexually, asexual reproduction, through fission or budding, also occurs in many families including spionids, cirratulids, syllids, and sabellids (Barnes, 1980). In the spionid *Pygospio elegans,* asexual reproduction occurs through transverse fission of the parent body into fragments, each of which will regenerate "lost" body regions (Rasmussen, 1953). Asexual reproduction has been widely reported in *P. elegans,* with most authors reporting its occurrence or testing environmental factors that may influence rates of division (Anger, 1984; Wilson, 1985). Despite the prevalence of asexual reproduction in this species, morphogenesis during post-fission regeneration has not been described.

Pygospio elegans is a tubiculous polychaete that is common on mud and sand flats and has a cosmopolitan, temperate distribution (Anger, 1984; Wilson, 1985). Adults grow to be 12 mm long, and feed on detritus (Wilson, 1985) and phytoplankton (Anger *et al.*, 1986). Rasmussen (1953) first described asexual reproduction in this species. He reported that both females and males could divide anywhere in the body and generally formed three to four fragments. Each fragment stayed in the original tube until regeneration was complete, about 8 d after division (20°C). Subsequently, several authors reported asexual reproduction in *P. elegans* from populations from the eastern seaboard of the United States (Hobson and Green, 1968), Washington State (Wilson, 1985), and the Baltic Sea (Anger, 1984; Gudmundsson, 1985). *P. elegans* also reproduces sexually, and it exhibits considerable flexibility in reproduction, as both planktotrophic and adelphophagic (a form of lecithotrophy) larval development have been reported in worms from different populations (*e.g.*, Thorson, 1946; Hannerz, 1956; Hobson and Green, 1968; Anger, 1984; Anger *et al.*, 1986; Schlötzer-Schrehardt, 1991; Morgan *et al.*, 1999).

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Our objective is to describe morphogenesis during post-fission regeneration in *P. elegans*. We use bright field and scanning electron microscopy (SEM) to describe morphogenesis after spontaneous divisions to determine (1) if anterior and posterior body regions show similar patterns of regeneration; and (2) how subsequent growth occurs. We use the term *regeneration* to refer to the replacement of lost body regions (*e.g.*, the head, thorax, tail) and *growth* as the addition of setigers to increase size, once the major body regions have formed. Also, we examine the rates of fission and fragment mortality in laboratory-maintained worms originating from three populations. No sexual reproduction was observed during the present study.

Materials and Methods

Adult specimens of *Pygospio elegans* were collected between May and September from three sites: Bon Portage Island, Starr's Point, and Conrad's Beach, Nova Scotia. At each site, sediments containing worms were sieved (500-μm mesh), and tubes were brought into the laboratory. *P. elegans* was identified following Bromley and Bleakney (1984). Worms were placed in either 250-ml Pyrex crystallizing dishes or 150-ml custard dishes, with seawater and defaunated sand. Dishes containing stock cultures were submerged in larger trays of seawater and aerated. Cultures were maintained at 20°C on a photoperiod of 16 h light. Worms were fed a mixture of dehydrated, ground *Enteromorpha* and Tetramin fish food suspended in seawater twice weekly. Seawater was changed once a week.

Stock cultures were sieved daily, and worms were isolated if they could be identified as having divided on that day (presence of a clean, smooth blastema) or showed signs that fission was about to occur (constriction of the body wall). Isolated worms were cultured separately to prevent movement of worms among culture dishes. Regeneration was observed with bright field and scanning electron microscopy. Fragments, anesthetised in 7% $MgCl_2$, were examined and photographed daily from fission to the completion of regeneration (8 d post-fission) using bright field techniques (n = 25 worms). Fragments at each stage of regeneration (2 to 3 fragments per stage for both anterior and posterior fragments) were prepared for SEM by fixation in 2.5% glutaraldehyde followed by post-fixation in 1% osmium tetraoxide, both in 0.1 *M* cacodylate buffer and seawater (Gibson *et al.*, 1999). After fixation, regenerates were dehydrated in an ascending series of ethanol, critical point dried with a Bio-Rad E3000 critical point drier, coated with gold-palladium with a Hummer II sputter coater, and observed with a JEOL JSM-25S or JEOL T330A scanning electron microscope. Growth was followed in additional worms that had completed the regeneration process (n = 12 worms). After the head and thorax or tail and pygidium had been regenerated, growth was examined by counting the number of setigers in each body region for a 17-d period.

Intact worms that showed no signs of a recent asexual event were cultured in isolation to determine rates of regeneration. Worms were observed from Bon Portage Island (n = 15), Starr's Point (n = 15), and Conrad's Beach (n = 10). Dishes were sieved weekly over a 6-week period. Original worm size was determined as the number of setigers at the beginning of the experimental period. Each week, the number of fragments per dish was noted, as well as the size of the fragments (number of setigers) and the degree of regeneration. Data were compared among the three study populations using one-way ANOVA in Statworks 1.2 (Cricket Software). Where significant differences were noted, a post-hoc Scheffé comparison was also performed using SPSS 8.0 (SPSS Inc.).

Results

Adult morphology

The overall body plan of *Pygospio elegans* is divided into four regions: the head, thorax, abdomen, and tail. The *head* is characterized by two ciliated palps, a prostomium with two or three pairs of eyes and paired nuchal organs (Fig. 1a). The *thorax* contains 10 to 12 abranchiate setigers, each with a single dorsal ciliary band, capillary notochaetae, and a lateral tuft of cilia. Neurochaetae are simple capillary on setigers 1 to 8 and hooded hooks on setigers 9 to 12 (Fig. 1b). The *abdomen* is 25 to 35 setigers in length. Each abdominal segment has paired branchiae and either a single (first few abdominal setigers) or double ciliary band, with two closely apposed bands of tufted cilia. Abdominal setigers also have capillary notochaetae, a lateral tuft of cilia, and neurochaetae that are hooded hooks (Fig. 1c). The *tail* contains 6 to 12 abranchiate setigers. Tail setigers have capillary notochaetae, neurochaetae that are hooded hooks, and a lateral tuft of cilia. There is a reduced ciliary band on the first few tail setigers only. The pygidium consists of four cirri, each with tufts of cilia on the inner surface (Fig. 1d). Male *P. elegans* have a pair of branchiae on the second setiger (Fauchald, 1977) and dorsal organs on each setiger (Schlötzer-Schrehardt, 1991). Only four males (n = 200 worms) were observed during the present study. No morphological differences were noted (SEM) between worms from the three study populations.

Morphogenesis following fission

In all cases, fragments retained their original anterior-posterior polarity. Posterior fragments regenerated only the head and thorax, and anterior fragments regenerated only a new tail and pygidium. Subsequent growth involved elongation of the tail by the addition of terminal setigers. We based our description on division into two fragments, as that

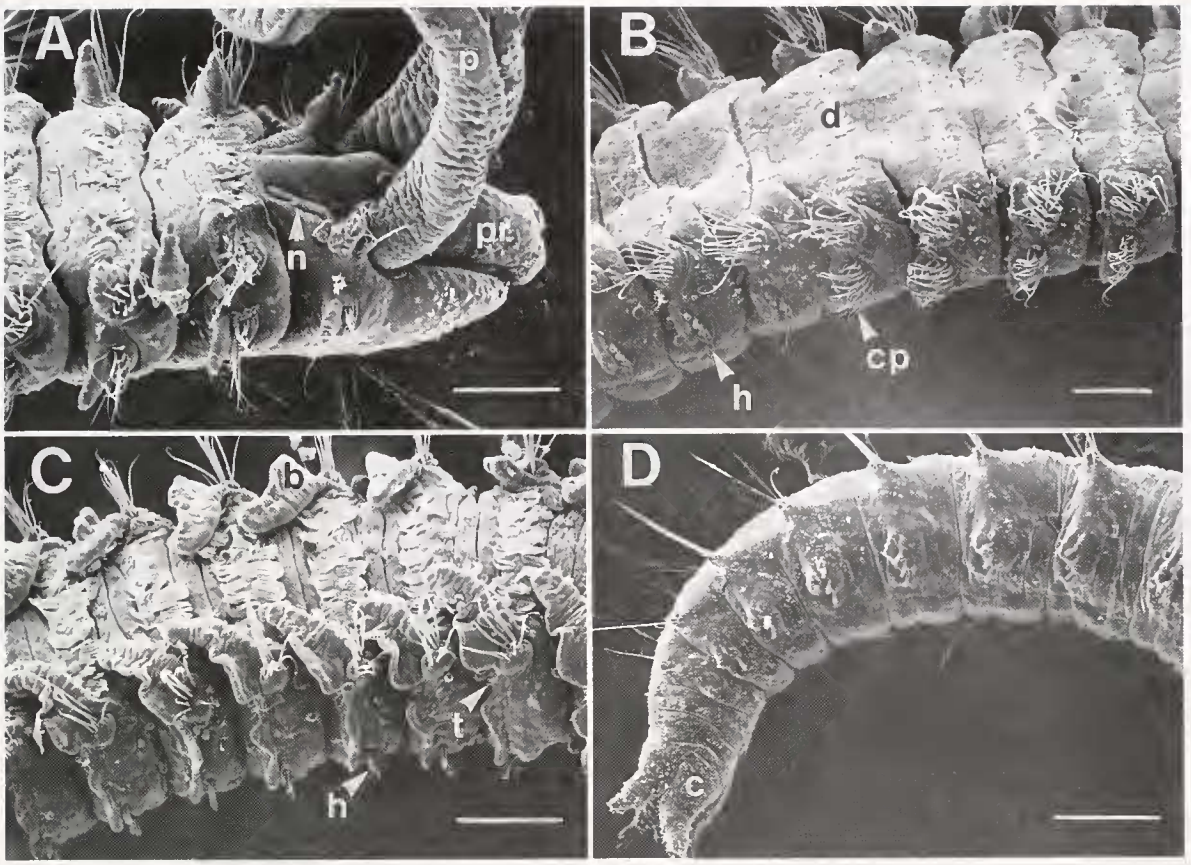

Figure 1. Scanning electron micrographs of adult *Pygospio elegans*. (A) Head and anterior thorax including the reduced first setiger. (B) Thorax, showing both anterior setigers with capillary neurochaetae and posterior setigers with neurochaetae that are hooded hooks. (C) Abdomen, characterized by branchiate setigers and a double dorsal ciliary band. (D) Tail and pygidium. b = branchus, c = cirrus, d = dorsal ciliary band, h = hooded hook, n = nuchal organ, cp = capillary chaetae, p = palp, pr = prostomium, t = tuft of cilia. Scale bar = 100 μm.

was the most common form of fission observed in the present study. The maximum number of fragments observed per division was six, and regeneration in all fragments followed the same basic pattern. Table 1 provides a list of the structures that were observed during regeneration and the time at which the regenerated structures were first observed.

On day 1, transverse fission began as a muscular constriction in the body wall, usually in the abdominal region located at a point about two-thirds along the length of the worm. Constriction of the body wall continued until the gut separated and the two fragments, each anchored to the substrate *via* mucous, pulled apart. The anterior fragment consisted of the head, thorax, and most of the abdomen (about 25 or more pairs of branchiae), while the posterior fragment consisted of the tail, pygidium, and usually about five or fewer branchiate abdominal setigers. The epidermis healed quickly and formed a smooth surface the same day as division occurred (Fig. 2a). On day 2, the blastema of both the anterior and posterior fragments showed a small amount of new tissue with tiny, scattered tufts of cilia on an otherwise smooth epidermis (Fig. 2b).

Regeneration on day 3 is characterized by rapid development of the blastema and formation of lost body regions (Table 1). As the anterior blastema increases in size, the regenerated head and thorax are readily distinguished (Fig. 2c). The head has palp buds, small dorsal depressions indicating formation of the nuchal organs, and a slightly rounded prostomium. The thorax shows the initial formation of 3 to 6 setigers, visible with both SEM and bright field microscopy. The gut, visible with bright field microscopy, has extended into the thorax near the parental abdomen. The tail blastema is smaller than the anterior blastema and shows 2–3 slight wrinkles, suggesting early segmentation. Bright field microscopy also revealed the formation of segments and as well as the extension of the gut into the tail region. Cirri buds are also visible (Fig. 2d).

On day 4, regeneration is characterized by further segmentation and early differentiation of region-specific structures. The anterior blastema has 8 to 12 well-defined seti-

Table 1

Summary of morphogenesis during regeneration in Pygospio elegans

Structure	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Anterior blastema		+						
Head			+					
Eyes (no. pairs)						1–2	2–3	2–3
Mouth				*	**	**	**	**
Nuchal organ			*	**	**	**	**	**
Palps			*	**	**	**	**	**
Prostomium			*	**	**	**	**	**
Thorax			+					
Setigers (no.)			3–6	8–12	10–12	10–12	10–12	10–12
Notopodial lobe					*	**	**	**
Notochaetae					*	**	**	**
Ciliary tuft					*	**	**	**
Neuropodial lobe					*	**	**	**
Neuropodial capillary chaetae						*	**	**
Neuropodial hooded hooks						*	**	**
Posterior blastema		+						
Tail			+					
Setigers (no.)			2–3	3–6	3–6	5–7	5–7	5–7
Notopodial lobe					*	**	**	**
Notochaetae					*	**	**	**
Ciliary tuft					*	**	**	**
Neuropodial lobe					*	**	**	**
Neuropodial hooded hooks						*	**	**
Pygidium			+					
Cirri			*	**	**	**	**	**
Ciliary tufts				*	**	**	**	**

+ = body region recognizable, * = structure visible as a bud or rudiment, ** structure well developed but smaller than in parent.

gers in the thorax (Fig. 2e, f), each with two dorsal tufts of cilia. The mouth and prostomium are visible on the regenerating head, and the nuchal organs have small cilia. The gut has extended from the original abdomen to the head (Fig. 2f). Segments are further developed in the posterior blastema as well, with 3 to 6 well-defined setigers, each with paired lateral pits in the region of the presumptive noto- and neurochaetae. Differentiation of the pygidium involves extension of the cirri and the appearance of small tufts of cilia on the inner surface (Fig. 2g).

On the fifth day post-fission, the anterior blastema has regenerated the entire thoracic region and shows early differentiation of segment-specific structures. The number of thoracic setigers (10 to 12) that regenerated in the anterior blastema is similar in all specimens regardless of where fission occurred in the parent worm. The head has an elongate prostomium. The thoracic setigers develop neuropodial and notopodial buds, with a few small capillary notochaetae and a small tuft of cilia between the neuropodium and the notopodium (Fig. 3a). The gut extends through the thorax, and the mouth is complete (Fig. 3b). On the same day, the 3 to 6 setigers of the posterior blastema also develop parapodial buds, a few notopodial capillary chaetae on setigers nearest the abdomen, and small lateral tufts of cilia. The pygidium has larger cirri with tufts of cilia (Fig. 3c).

Regeneration on day 6 involves greater differentiation of segment-specific structures and addition of posterior setigers to restore the parental organization of the tail. The regenerated head has elongate, ciliated palps, a blunt prostomium (Fig. 3d), and 1 to 2 pairs of subdermal eyes (Fig. 3e). The thorax has dorsal bands of cilia on each setiger and well-developed notopodial chaetae throughout. Also in the thorax, the neuropodia exhibit short capillary chaetae on setigers 1–8 and a single hooded hook per setiger from setiger 8 posteriorly. The tail blastema has the 5 to 7 setigers characteristic of this region, with capillary notochaetae and notopodial hooded hooks that decrease in number from three on the proximal, earliest-forming setiger, to one on the later-developing terminal setiger (Fig. 3f). Lateral tufts of cilia are present on all setigers. The pygidium has cirri that are mature in size and have well-developed tufts of cilia.

By day 7, the anterior blastema has regenerated a head and thorax that are identical to those of the parent worm except in setiger size and number of chaetae (Fig. 3g). Subsequent development in this region involves an increase in setiger size but not number. In the tail, setiger size and chaetae number also increases (Fig. 3h). By day 8, the

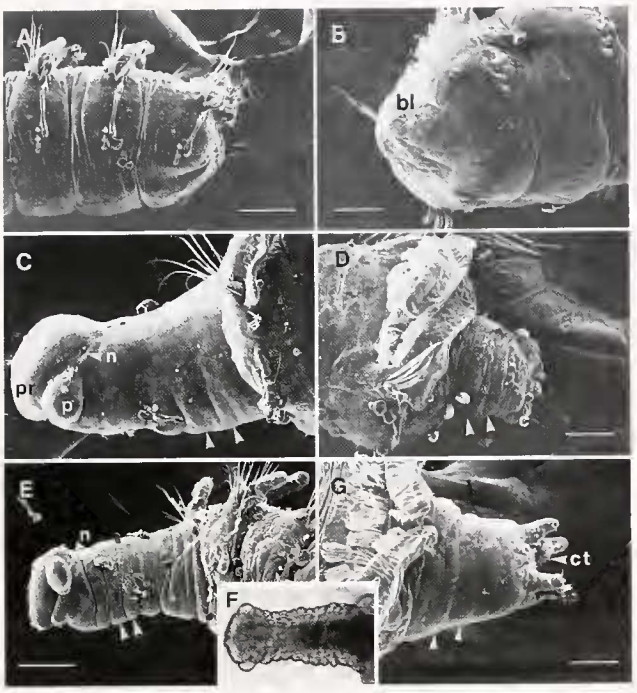

Figure 2. Early regeneration in *Pygospio elegans* following spontaneous transverse fission. (A) Day 1 post-fission, anterior fragment. (B) Day 2, posterior fragment with blastema. (C) Day 3, anterior blastema, showing regenerated head, thorax, and evidence of early segmentation. (D) Day 3, posterior blastema, showing regenerated tail and buds of cirri. (E) Day 4, regenerated head and thorax with segments. The head has a regenerated mouth, palp buds, and a rounded prostomium. (F) Day 4, regenerated head and thorax showing extension of the gut into regenerated tissue. (G) Day 4, regenerated tail and pygidium. A–E, G are scanning electron micrographs, F is a bright field micrograph. bl = blastema, c = cirri bud, n = nuchal organ, p = palp bud, pr = prostomium, t = tuft of cilia. Arrows indicate setigers. Scale bar = 100 μm for A and E, 50 μm for B–D and G.

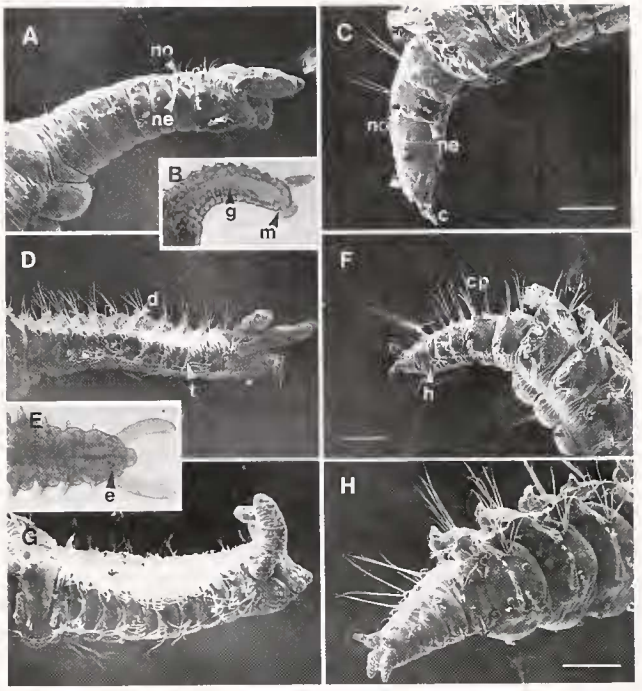

Figure 3. Completion of regeneration in *Pygospio elegans*. (A) Day 5 post-fission, anterior regenerate. (B) Day 5, anterior regenerate showing development of the gut, mouth, and setigers. (C) Day 5, posterior blastema. (D) Day 6, anterior regenerate. (E) Day 6, regenerated head with two pairs of eyes. (F) Day 6, regenerated tail and pygidium. (G) Day 7, anterior regenerate. (H) Day 7, posterior regenerate. A, C, D, F–H are scanning electron micrographs. B and E are bright field micrographs. c = cirrus, cp = capillary chaetae, d = dorsal ciliary band, e = eyes, g = gut, h = hooded hooks, m = mouth, ne = neuropodium, no = notopodium, t = tuft of cilia. Scale bar = 100 μm, anterior and posterior fragments for each day are shown at the same magnification.

regenerated thorax and tail have an increased number of chaetae, and are similar to the pre-fission organization except for setiger size. Also on day 8, the gut extends through the new tail to the pygidium.

In all fragments, regeneration produces only specific body regions, regardless of where fission occurred in the parent. Anterior fragments regenerate only the pygidium and the 6 to 12 abranchiate setigers of the tail. Posterior fragments regenerate only the thorax (10 to 12 setigers) and head. Mid-worm fragments concurrently regenerate both anterior and posterior regions as described above, with the result that these fragments regenerate the head and thorax and tail and pygidium but not the abdomen, regardless of the size of the original fragment (Fig. 4). After regeneration, worms grow to their pre-fission size by increasing setiger size and setiger number. During the growth phase, new setigers will only form immediately anterior to the pygidium; new setigers do not form in the thorax or abdomen once regeneration is complete. Newly formed terminal setigers develop chaetae and parapodial lobes typical of the

Figure 4. Scanning electron micrograph of a specimen of *Pygospio elegans* regenerating from a mid-worm fragment, about 6 days after fission. The larger, parental setigers originated from the abdominal region and have branchiae. Both the anterior (head, thorax) and posterior (tail, pygidium) regions have regenerated. Scale bar = 100 μm.

tail region (Fig. 5). As the tail region increases in setiger number, anterior tail setigers differentiate into abdominal setigers by forming dorsal ciliary bands and branchiae buds. About one-half of a setiger is added each day during the growth phase (n = 11 worms, mean ± SD 0.52 ± 0.23).

Occasional anomalies were noted in this general pattern. For example, Figure 6 shows a *P. elegans* that regenerated two thoracic regions and heads, both containing extensions of the gut. Such anomalies, although rare, reinforced the general pattern of regeneration described above. For both heads, the blastema gave rise to a specific number of setigers, and segmentation was followed by differentiation.

Population comparison

Frequency of spontaneous division and mortality were compared in *P. elegans* originating from the three populations. Specimens from all three populations were roughly the same size at the start of the experiment and ranged from 25 to 62 setigers overall (Table 2). Worms from Starr's Point and Conrad's Beach divided about once per week (1.3 and 1.2 weeks between divisions, respectively), while worms from Bon Portage Island divided less frequently (3.6 weeks between divisions; Table 2), although sample sizes were low for the Starr's Point and Bon Portage Island worms. Most worms divided into two fragments, but up to six fragments per division were observed. Conrad's Beach worms divided at the smallest size (average of 34 setigers), whereas those from Starr's Point and Bon Portage were, on average, larger before undergoing fission (42 and 45 setigers, respectively; Table 2).

Mortality was also compared among regenerating fragments. Fragments were classified according to the remaining original body region into anterior (containing the original head, thorax, and anterior abdomen), mid-worm (abdominal setigers only), and posterior fragments (original pygidium, tail, and a few abdominal setigers). Anterior and mid-worm fragments had a relatively low mortality, approximately 14% for all three populations combined (Table 2), although few mid-fragments were observed because most worms spontaneously divided into two fragments (anterior, posterior) only. Posterior fragments had the highest mortality overall, about 80% among all three populations (Table 2).

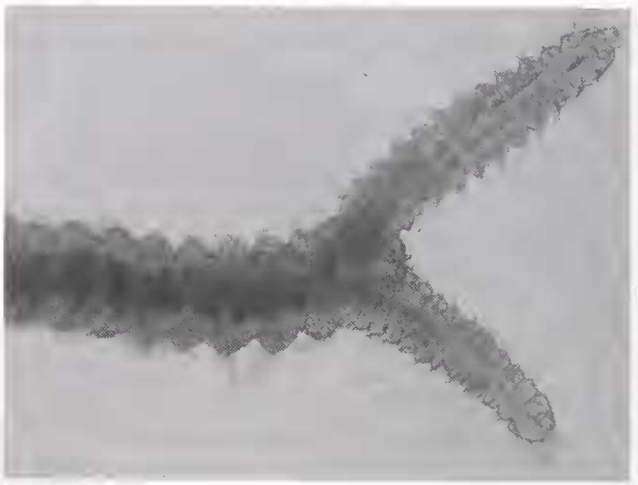

Figure 6. Bright field micrograph of a two-headed individual of *Pygospio elegans*, formed after a spontaneous asexual event.

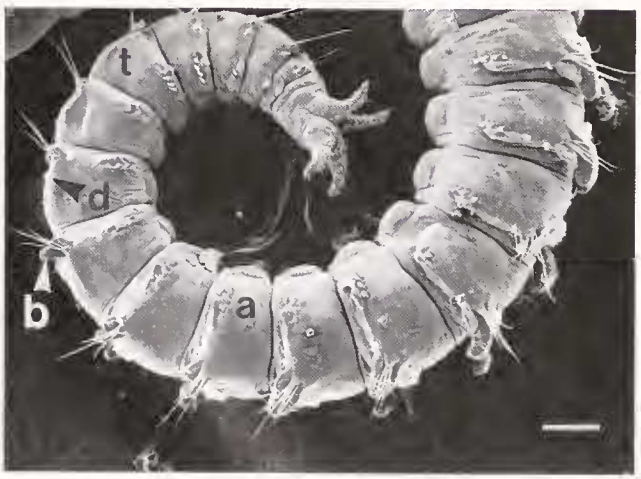

Figure 5. Growth in *Pygospio elegans*. Growth occurs through the addition of terminal setigers, immediately anterior to the pygidium. New setigers develop the parapodial lobes and chaetae characteristic of the tail (t). Transitional setigers show branchiae buds (b) and tufts of dorsal cilia (d) as they gradually differentiate into abdominal setigers (a). Scale bar = 100 μm.

Discussion

Morphogenesis after asexual reproduction in *Pygospio elegans* involved two phases: regeneration of lost body regions (*e.g.*, head, thorax, tail) followed by appositional growth as terminal setigers were added. During regeneration of the anterior region, the blastema extended to form the head and thorax, segmented to divide the thorax into 10–12 setigers, and subsequently developed segment-specific structures (*i.e.*, chaetae). Regeneration of the posterior blastema was similar and also involved formation of a finite, though variable, number of setigers (6 to 12). The origin of blastemal tissues was not examined in the present study, but probably involves the growth of existing tissues (*e.g.*, epidermis) in combination with the migration of mesodermal—and possibly endodermal—neoblasts, as occurs in other annelids (Hill, 1970; Christensen, 1994).

Once the thorax or tail had become reestablished, growth occurred but was restricted to a growth zone immediately anterior to the pygidium; new setigers did not appear elsewhere in the body. Growth by the formation of terminal setigers is common in spionid adults and larvae. The abdo-

Table 2

Asexual reproduction in Pygospio elegans

Trait	Population			ANOVA
	Bon Portage	Starr's Point	Conrad's Beach	
Original size (no. setigers)	42.1 ± 3.2 n = 15	35.4 ± 1.2 n = 15	41.6 ± 3.0 n = 10	$F_{(2,39)} = 2.2$, $P = 0.12$
Fission				
No. Weeks between divisions	3.6 ± 0.5 n = 5	1.3 ± 0.3 n = 3	1.2 ± 0.1 n = 21	$F_{(2,28)} = 26$, $P = 0.000$ CB = SP < BP
Size at division (no. setigers)	44.6 ± 3.3 n = 17	41.5 ± 2.1 n = 11	34.0 ± 1.9 n = 26	$F_{(2,53)} = 5.5$, $P = 0.007$ CB < BP, SP ns
No. Fragments per division	2.2 ± 0.1 n = 17	2.2 ± 0.1 n = 11	2.7 ± 0.2 n = 26	$F_{(2,52)} = 2.4$, $P = 0.10$
Mortality (no. dead/no. fragments per type)				% Mortality
Anterior fragment	6/26	0/11	3/29	13%
Middle fragment	1/2	1/2	2/24	14%
Posterior fragment	14/14	7/8	11/18	80%

Data are means, standard errors, and sample sizes (n) for traits indicated, in a comparison between laboratory-maintained worms from three populations. The final column gives results of a one-way ANOVA among populations and results of a post-hoc Scheffé comparison among populations, where significant differences were found.

men increased in size only during the growth phase as tail setigers differentiated into abdominal setigers by developing branchiae and dorsal cilia. In all fragments, regeneration produced only specific body regions, regardless of where fission occurred in the parent. For example, one worm divided in the original thoracic region and, after fission, had only a head and nine thoracic setigers. This individual regenerated only a tail and pygidium; abdominal setigers redifferentiated from tail setigers as growth proceeded. Many worms were observed to undergo a second asexual event before growth was complete (often 8 to 10 days after fission), and several individuals divided almost immediately after fission (days 1–3) as evidenced by the presence of fragments at different stages of regeneration in a single culture.

Spionids, in general, are not as well known for their ability to regenerate as are some other polychaetes, such as sabellids. Asexual reproduction by regeneration is common, however, in the spionids *Polydora tetrabranchia* (Campbell, 1955) and throughout the genus *Polydorella* (Radashevsky, 1996). In *Polydorella,* unlike *P. elegans,* new individuals are formed by paratomy, resulting in a chain of clones. Otherwise, morphogenesis during an asexual event in *Polydorella dawydoffi* is similar to architomy in *P. elegans:* the new individual forms through development of a growth zone (similar to the blastema reported here), elongation to form specific anterior body regions (*i.e.,* head and thorax), segmentation resulting in a specific number of thoracic setigers, and differentiation to form region-specific structures such as the chaetae, eyes, and branchiae. Once the new head has formed in *Polydorella,* transverse fission occurs and the two daughter worms separate (Radashevsky, 1996). Although asexual reproduction does not appear to be widespread in spionids, regeneration as a response to tissue loss (*e.g.,* palps or the tail) occurs frequently in *Polydora cornuta* (Zajac, 1985, 1995), *Boccardia proboscidea* (Gibson, pers. obs.), and *Streblospio benedicti* (Harvey, pers. obs.). Further work may reveal whether the restricted potential for asexual reproduction within the spionids could have arisen by decoupling regeneration and reproduction, as has been suggested in the oligochaete *Paranais litoralis* (Bely, 1999).

Although the mechanisms leading to the restoration and differentiation of body regions are not known, it seems likely that the regulatory genes important in embryogenesis may play a role. For example, *distal-less* is known to be important in the development of parapodia in polychaete embryos (Panganiban *et al.,* 1997) and possibly is reactivated during regeneration, although this remains to be demonstrated. In an asexual race of *Dugesia tigrina* (platyhelminth), lost body regions are defined during regeneration by Hox genes that have sequences very similar to those found in annelids (Bayascas *et al.,* 1998). Interestingly, in this race of *D. tigrina,* Hox genes were found to be permanently expressed in adults, perhaps contributing to the impressive regenerative capabilities of this species (Bayascas *et al.,* 1998).

There were no differences among populations in original size and number of fragments per asexual event in *Pygospio elegans,* although time between divisions and size at division did vary. Rasmussen found that rates of division increased at low temperatures, and Wilson (1985) found that

division rates increased at low worm densities. Anger (1984) observed that the number of individuals (in an asexual population) increased at low salinity and temperature. In the present study, fission was observed in isolated worms that were maintained under constant conditions (34 ppt, 20°C and with an abundance of food); therefore, these conditions were unlikely to contribute to the differences in division we observed among laboratory cultures.

Posterior fragments (original tail) had a higher mortality than did anterior fragments (original head). Posterior fragments were much smaller than anterior fragments; had few branchiate, abdominal setigers (5–6 on average, *vs.* 25 for anterior fragments); and lacked a mouth until day 5 post-fission and therefore were unable to feed immediately after division. Differences in mortality could be due to fragment size (*e.g.,* energy reserves or number of neoblasts available) or lack of a mouth. However, the few mid-worm fragments observed during the present study had a high survivorship, despite their small size. Despite the high mortality of posterior fragments, extensive laboratory culturing by others indicates a net population growth through asexual reproduction (Anger, 1984; Wilson, 1985).

Although *P. elegans* is known to reproduce sexually (Thorson, 1946; Hannerz, 1956; Anger, 1984; Anger *et al.,* 1986; Morgan *et al.,* 1999), only asexual reproduction was noted in the worms observed in the present study (more than 200 in total). This suggests that asexual reproduction is the dominant reproductive mode in these populations during the study period (May–September). Anger (1984) reported a population in the Kiel Bight, Baltic Sea, that reproduces exclusively through asexual reproduction; two additional populations were predominantly sexual, although occasional fragmentation was noted. Anger (1984) attempted to induce specimens of *P. elegans* from these three populations to switch between sexual and asexual reproduction by varying culture conditions (temperature and salinity) but found that worms retained the reproductive mode of their original population, leading her to suggest the potential for cryptic species. Other investigators have reported seasonal differences in reproductive mode within a single population, with asexual reproduction being dominant in the spring or summer, and sexual reproduction prevalent in the fall or winter (Rasmussen, 1973; Hobson and Green, 1968; Wilson, 1985). Rasmussen (1953) also noted that fission could be induced in *P. elegans* by temperatures of 4°–5°C.

In addition to asexual reproduction, *P. elegans* exhibits considerable flexibility in sexual reproduction, including both planktotrophic and adelphophagic larval development (*e.g.,* Thorson, 1946; Hannerz, 1956; Hobson and Green, 1968; Anger, 1984; Anger *et al.,* 1986). This suggests the potential for reports of *P. elegans* to include cryptic species, but Morgan *et al.* (1999) clearly demonstrated that poecilogony does exist in this species, based on a molecular (allozyme) comparison of populations with planktotrophic or adelphophagic development. Poecilogony in *P. elegans* is, in several regards, similar to that of the spionids *Boccardia proboscidea* (Blake and Kudenov, 1981; Gibson, 1997) and *Polydora cornuta* (MacKay and Gibson, 1999), which also reproduce by means of planktotrophic and adelphophagic larval development. Such flexibility makes *P. elegans* a valuable model for tests of the ecological consequences of life-history variability, as well as for understanding the developmental mechanisms underlying a change in development mode.

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